

hexamer primers in a 10 μ l reaction by heating to 70° C. for 10 minutes followed by 2 minutes on ice. cDNA is then synthesized by adding 1 μ l of RNAsin (Promega, Madison Wis.), 2 μ l of 50 mM dNTP's, 4 μ l of 5X buffer, 2 μ l of 100 mM DTT and 1 μ l (200 U) of Superscript II™ AMV reverse transcriptase. Random primer, dNTP's and Superscript II™ reagents are all purchased from Life-Technologies, Gaithersburg, Mass. The reaction is incubated at 42° C. for 1 hour. Amplification of p38 cDNA is performed by aliquoting 5 μ l of the reverse transcriptase reaction into a 100 μ l PCR reaction containing the following: 80 μ l dH₂O, 2 μ l 50 mM dNTP's, 1 μ l each of forward and reverse primers (50 pmol/ μ l), 10 μ l of 10X buffer and 1 μ l Expand™ polymerase (Boehringer Mannheim). The PCR primers incorporated Bam HI sites onto the 5' and 3' end of the amplified fragment, and are purchased from Genosys. The sequences of the forward and reverse primers were SEQ ID 1:
5'-GATCGAGGATTCTCATGTCTCAGGAGAGGCCA-3' and SEQ ID 2:
5'GATCGAGGATTCTCAGGACTCCATCTCTTC-3' respectively. The PCR amplification is carried out in a DNA Thermal Cycler (Perkin Elmer) by repeating 30 cycles of 94° C. for 1 minute, 60° C. for 1 minute and 68° C. for 2 minutes. After amplification, excess primers and unincorporated dNTP's is removed from the amplified fragment with a Wizard™ PCR prep (Promega) and digested with Bam HI (New England Biolabs). The Bam HI digested fragment is ligated into BamHI digested pGEX 2T plasmid DNA (PharmaciaBiotech) using T-4 DNA ligase (New England Biolabs) as described by T. Maniatis, Molecular Cloning: A Laboratory Manual, 2nd ed. (1989). The ligation reaction is transformed into chemically competent E. coli DH10B cells purchased from Life-Technologies following the manufacturer's instructions. Plasmid DNA is isolated from the resulting bacterial colonies using a Promega Wizard™ miniprep